



US serial No. 09/603,885  
Applicant's docket No. 004

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re of the Application of

Stephen Michnick et al

Serial Number: 09/603,885

Filed: June 26, 2000

For: AN IN VIVO LIBRARY VERSUS  
LIBRARY SELECTION OF OPTIMIZED  
PROTEIN-PROTEIN INTERACTIONS

Group Art Unit: 1627  
Examiner: B. Celsa

**RESPONSE TO OFFICE ACTION DATED SEPTEMBER 24, 2001**

Hon. Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

Dear Sir:

In response to the office action dated September 24, 2001, it is respectfully submitted that the present application as filed does not need a sequence listing as required by 37 C.F.R. 1.821-1.825.

The present invention describes a strategy for library-vs-library screening in intact cells based on the folding of murine enzyme dihydrofolate reductase (mDHFR) from complementary fragments. DHFR is genetically dissected into two rationally designed fragments, each of which can be fused to a library of proteins or peptides (Fig. 1A). Members of one library which heterodimerize with a member of the other library drive the reassembly of the mDHFR fragments, resulting in reconstitution of enzymatic activity (Fig. 1B). Activity is detected *in vivo* using an *E. coli*-based selection

assay, where the bacterial DHFR is specifically inhibited with trimethoprim, preventing biosynthesis of purines, thymidylate, methionine and pantothenate, and therefore cell division. The reconstituted mDHFR, which is insensitive to the low trimethoprim concentration present in selection, restores the biosynthetic reactions required for bacterial propagation. As a result, the interaction between library partners is directly linked to cell survival and detected by colony formation.

As explained in the Examples (See Example 1 in page 21) of the invention "...The DNA constructs encoding the *N*-terminal (1-107) and *C*-terminal (108-186) mDHFR fragments have been previously described (see reference 5). Briefly, each fragment was amplified by PCR with appropriate unique flanking restriction sites and subcloned into a bacterial expression vector (pQE-32 from Qiagen)..."

In its broadest aspect, the present invention is directed to a method for identifying an interacting set of molecules comprising:

- A) generating fragments of a reporter molecule which have a directly or indirectly detectable activity when associated;
- B) coupling first fragments to members of a first panel of molecules;
- C) coupling second fragments to members of a second panel of molecules;
- D) mixing the products of B) and C);
- E) directly or indirectly testing for said activity; and
- F) identifying the panel members whose interaction resulted in said activity and which thus form an interacting set.

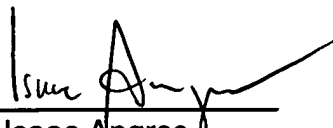
The generation of fragments is well described in the specification as well as in references cited therein.

Additionally, applicant has been recently granted two U.S. Patents on the basic and pioneering technology which utilizes DHFR (dihydrofolate reductase) fragments: U.S. Nos. 6,270,964 and 6,294,330 (copies are enclosed herewith for the Examiners benefit as well as to fulfill duty of disclosure under 37 C.F.R. 1.56 although). It should be noted that in neither of those two patented cases was there a need to file sequence listings.

In view of the above, it is respectfully requested that the notice to comply with the requirements of 37 C.F.R. 1.821-1.825 be withdrawn.

In view of the above, an early action on the merits are courteously requested.

Respectfully submitted,

  
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